

COMPARATIVE STUDIES OF THE METABOLISM OF
AMINO ACIDS.

By ROBERT HUGH WILSON.

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIRE-
MENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE
UNIVERSITY OF MICHIGAN.

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COMPARATIVE STUDIES OF THE METABOLISM OF AMINO ACIDS.

II. THE RATE OF ABSORPTION OF AMINO ACIDS FROM THE GASTROINTESTINAL TRACT OF THE WHITE RAT.*

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The change in view-point which followed the discovery that protein is hydrolyzed to amino acids in the intestinal tract, and is absorbed and transported in this form, has made necessary an intensive study of the behavior of the individual amino acids, components of the protein molecule, in the organism, if a true picture of protein metabolism is to be obtained. This shift in view-point has tended to simplify the study of protein metabolism since the various proteins, even those of widely different physical properties, present, when hydrolyzed, essentially the same qualitative picture. The problem thus is altered from a consideration of many compounds (the individual proteins) to a consideration of relatively few (the amino acids).

Studies of the intermediary metabolism of the individual amino acids have been concerned, for the most part, with the results of urine analysis, or in a more limited series of investigations, with the results of the analysis of the blood, following ingestion of amino acids. These have indicated the paths which may be traveled by these substances after they enter the blood stream and the end-products of the reactions of their metabolism. Time relationships, however, are indefinite. There are indications that not all amino acids are metabolized at the same rate or with equal

* The material presented here represents an abstract of part of the thesis presented by Robert Hugh Wilson in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

ease and that of the various reactions involved, some are rapid, others slow. A careful comparative study of the rates of metabolism of amino acids is still lacking. Under normal conditions of nutrition, one of the first variables of significance in the chain of events involved in the actual metabolism of the amino acids would be the rate at which each enters the blood stream from the intestine.

In previous studies by one of us (1), differences in the partition of the non-protein nitrogen of the blood, following oral administration of various amino acids, were observed in rabbits. It was suggested that the differences observed might be explained, in part, at least, by variations in the rate at which the amino acids were absorbed from the gastrointestinal canal. Direct proof of this, however, could not be obtained in the rabbit. The experimental procedure recently developed by Cori (2) has now made possible such a study of the absorption of the amino acids with the white rat. The present investigation is concerned with the rates of absorption from the gastrointestinal tract of the albino rat of the amino acids, glycine, *d*-alanine, *dl*-alanine, *d*-glutamic acid, and *l*-leucine. Further studies to include other amino acids of different types are in progress.

EXPERIMENTAL.

The method of study has been well summarized by Cori (2): "The principle is briefly as follows: A known amount of the substance under investigation is fed by stomach tube. After a given time, the rats are killed and the amount of substance remaining in the intestines is determined quantitatively. The difference between the amount fed and amount recovered from the whole intestinal tract is then the amount of substance absorbed." White rats, ranging in weight for the most part from 100 to 200 gm., were used. They were maintained on a stock diet of whole wheat bread, milk, and lettuce for periods of 7 to 30 days. At the beginning of the experiments, they were fasted for 24 hours¹ (in a

¹ Since the rats were to be used for a study of the formation of glycogen from amino acids as well as for the study of rates of absorption, the shorter fasting period of 24 hours was considered advisable since Barbour, Chaikoff, Macleod, and Orr (3) had shown that the glycogen content of white rats was lower after a 24 hour fast than after a fast of 48 hours duration. The

few cases 48 hours). The amino acids were then fed by stomach tube, the amount administered being determined by measuring an equal amount of the solution used into a 50 cc. volumetric flask and subsequently determining the amino acid nitrogen content of the solution.

After a period of 1 to 4 hours, the animals were killed either by a blow on the head or by chloroform and after ligation of the esophagus and rectum, the gastrointestinal tract was removed. This was placed immediately in an ice chamber at freezing temperature, in which it remained until the material could be analyzed. An attempt was made to keep this period, elapsing before analysis, constant (3 hours) in all experiments. Blood samples were taken from the heart immediately after the death of the animal and the amino acid content was determined colorimetrically by the method of Folin.

The gastrointestinal tract was slit open and was thoroughly washed out with about 200 cc. of hot water. To the washings were added 5 cc. of 3 per cent acetic acid, which afforded an acidity optimum for heat coagulation and flocculation of the proteins.² The washings were heated to boiling in order to destroy any proteolytic enzymes present and to coagulate the proteins, allowed to stand a few minutes, and filtered by suction. To the filtrate were added 5 cc. of a 10 per cent solution of sodium tungstate, enough 0.67 N sulfuric acid to cause the appearance of a flocculent precipitate,³ and water to give a final volume of 500 cc. After 30 minutes, the precipitate was removed by filtration. To 400 cc. of the filtrate, sodium hydroxide was added until the reaction was only weakly acid to litmus, and the solution was concentrated to about 100 cc. on the steam bath. It was then made slightly alkaline with sodium hydroxide and boiled for 2 to 3 minutes to remove any ammonia present. The solution was made acid with

shorter fasting period seemed to have no effect on either the residual amino acid nitrogen of the intestine in the control animals or on the rate of absorption of glycine (*cf.* Tables I and II).

² The pH as measured colorimetrically in two experiments was observed to be 4.0 and 4.2.

³ Addition of a volume of 0.67 N sulfuric acid, equal to the volume of the sodium tungstate solution added, did not result in a precipitate which could be filtered readily. Somewhat more acid was required.

acetic acid, concentrated, and made up to a final volume of 50 cc. The amino acid nitrogen of the solution was determined according to the gasometric method of Van Slyke. The procedure used in preparing the material for analysis is similar to that used by Hiller and Van Slyke (4) in the determination of amino acid and peptide nitrogen of blood, a procedure which, as shown by them, did not effect hydrolysis of peptides. It was necessary to demonstrate that quantitative recovery of added amino acid nitrogen could be obtained by the above procedure. The washings from the intestines of two rats were combined, thoroughly mixed, and divided into two equal portions. The amount of amino nitrogen present was determined in the first portion. To the second was added a known amount of leucine;⁴ the proteins were then precipitated and the amino acid nitrogen determined as before. In two different experiments there was a loss in one case of 0.64 mg., and a gain of 0.46 mg. of amino nitrogen in the other, amounts which were well within the cumulative error of the three nitrogen determinations involved. Since of the amino acids studied leucine is least soluble, it is probable that similar experiments with the other amino acids would have shown recoveries equally satisfactory. These results confirm the work of Bock (5) and Hiller and Van Slyke (4), which showed that heat coagulation and tungstic acid precipitation did not precipitate the amino acids present in blood.

Residual Amino Nitrogen in the Intestine.

Before studying the rate of absorption of the amino acids, it was necessary to establish normal values for the amino nitrogen content of the gastrointestinal canal of the albino rat after short fasts. A series of control animals was treated in the same manner as were those fed the amino acids, except that the control animals received 2 cc. of distilled water and were killed shortly thereafter. The results obtained with these control rats are presented in Table I. The residual amino acid nitrogen content of the intestine was found to be rather high, a result contrasting with the value obtained for the reducing substances of the intestine by Cori (2). In a verbal communication Cori has informed us that

⁴ The amounts of leucine added (140 to 180 mg.) were of the same order of magnitude as the amounts of leucine recovered from the intestine after a period of absorption in the leucine experiments.

he also has noted a fairly high residual amino acid figure, although the exact values were not given. In general it may be said that two fairly distinct groups of values were obtained; the first ten experiments recorded in Table I, which show an average value of 12.03 mg., were carried out the spring of 1928, and the remainder, designated as Series II, in the following autumn and winter. In the calculations of the amount of the amino acids remaining in the intestine after the period of absorption, this difference was taken

TABLE I.
Content of Amino Acid Nitrogen in the Intestine of Fasting Rats.

Series I.*					Series II.†				
Rat No.	Sex.	Length of fast.	Weight after fast.	Amino acid N.	Rat No.	Sex.	Length of fast.	Weight after fast.	Amino acid N.
		hrs.	gm.	mg. per 100 gm.			hrs.	gm.	mg. per 100 gm.
15	F.	48	187.0	14.0	63	M.	24	145.5	11.4
16	M.	48	116.0	8.6	64	"	24	92.0	6.9
17	F.	48	123.0	10.2	65	"	24	98.0	8.8
18	"	24	144.0	13.3	83	"	24	160.0	5.2
19	"	24	141.0	10.6	87	"	24	129.0	7.9
20	"	48	151.0	14.4	88	"	24	171.5	7.0
21	M.	48	194.0	15.0	89	"	24	130.5	7.6
22	"	48	193.0	10.2	99	"	24	117.0	3.8
23	"	24	94.0	10.0	123	"	24	162.0	9.7
24	"	24	108.0	14.0	124	"	24	183.5	7.5
					125	"	24	154.5	6.7
					126	"	24	163.5	6.5
Average				12.03	Average.....				7.42

* Determined during the spring of 1928.

† Determined during the autumn and winter of 1928-29.

into consideration, the amount subtracted being determined by the date of the experiment. Rats whose experimental number is lower than 59 belong to the same group as Series I (Table I) of the control animals.

*Glycine.*⁵—The rate of absorption of glycine (Table II) was determined for seventeen rats, the absorption periods ranging from

⁵ The product of the laboratories of the Eastman Kodak Company.

1 to 4 hours. An inspection of Table II shows that while the average rate of absorption was 53.1 mg. of glycine per 100 gm. per

TABLE II.
Rate of Absorption of Glycine.

Rat No.	Sex.	Weight after fast.*	Absorption time.	Glycine fed.	Glycine recovered.	Rate of absorption.
		gm.	hrs.	mg. per 100 gm.	mg. per 100 gm.	mg. per 100 gm. per hr.
9	M.	207.0	1	250	213	37
12	"	234.0	1	200	145	55
26	"	110.0	1	459	352	107
Average.....						66.3
10	F.	180.0	2	286	196	45
13	M.	222.0	2	208	104	52
27	"	116.0	2	440	333	54
98	"	111.5	2	315	198	59
Average.....						52.5
14	M.	206.0	3	221	46	58
28	"	114.0	3	452	285	56
101	"	97.0	3	362	202	53
102	F.	99.0	3	356	203	51
107	M.	166.0	3	321	194	42
108	"	156.0	3	260	124	45
Average.....						50.8
11	F.	172.0	4	315	116	50
100	M.	109.5	4	321	124	49
109	"	127.0	4	420	222	50
110	"	115.5	4	351	190	40
Average.....						47.3
" of all experiments.....						53.1
" " 2, 3, and 4 hr. experiments.....						50.3

* Rats 9 to 14 were fasted for 48 hours, the others 24 hours.

hour, the values ranged from 37 to 107 mg. If, however, the results obtained with the 1 hour absorption period are omitted, the average becomes 50.3 mg., with variations of only 40 to 58 mg.

per 100 gm. per hour. As will be shown later, a much greater variability would be expected during the shorter period, and for this reason the average value for the 2, 3, and 4 hour absorption periods is probably more nearly the true value.

At this point in order to make Tables II to VIII clear it may be desirable to illustrate the procedure by citing the details of a typical experiment. Rat 10 (Table II), a female weighing 180 gm., was fed a glycine solution containing 103.3 mg. (uncorrected) of amino acid nitrogen as determined by the Van Slyke method. Since this method gives values which are too high when glycine is used, this figure has to be corrected by multiplying by 0.93. As glycine contains 18.67 per cent nitrogen, the amount of glycine fed was $\frac{103.3 \times 0.93}{0.1867} = 515$ mg., or 286 mg. per 100 gm. The intestinal

contents contained 92.6 mg. of amino acid nitrogen, or 51.4 mg. per 100 gm. The experiment was carried out in February, 1928. Therefore the blank for the spring series, 12.03 (Table I), was subtracted from this, leaving 39.4 mg. of amino acid nitrogen due to the glycine fed or 196 mg. of glycine per 100 gm. (the factor 0.93 again being taken into consideration). The difference between the amount fed, 286 mg., and the amount remaining in the intestinal tract is the amount absorbed (90 mg.). Since the absorption took place over a 2 hour period, the rate of absorption was 45 mg. per 100 gm. per hour.

If we consider now the variations in rate of absorption during the 1 hour period, the reason, at least in part, for these can be seen. On the assumption that a certain rat has a residual nitrogen content of 8.6 mg. instead of 12.0 mg. per 100 gm., which has been shown in Table I to be quite possible, the amount of glycine not absorbed will actually be 16.9 mg. more than the value just computed. With only a 1 hour absorption period, this error would be transmitted bodily to the absorption rate column, while if the period had been longer, this error would have been divided by the number of hours, so that the determinations over the longer periods should show a greater similarity, as has been shown to be true. This variability due to unlike residual nitrogen contents, would be even greater when the nitrogen content of the amino acid fed is less than that of glycine, and for this reason, in some of the absorption experiments to be reported, the 1 hour absorption period has been eliminated entirely.

On the other hand, a determination for a period longer than 4 hours is not practicable because of the difficulty in obtaining a solution concentrated enough to allow absorption to proceed for a greater length of time, and because with the larger doses and particularly when the amino acid is fed as a salt, diarrhea often develops in a few hours. While, to a large extent, the variations in the rate of absorption of the shorter periods can be explained by the above reasoning, this is not entirely true, some of the values being outside of this range of variability.

Glycine as the Sodium Salt.—Because leucine and glutamic acid, two of the amino acids which were used for determinations of the rate of absorption, were too insoluble to make a solution as concentrated as was needed for this study when in an uncombined state, they were fed as sodium salts. It seemed possible that the introduction of the sodium ion might change the absorption rate of these acids, so, in order to see if this were the case, glycine to which had been added approximately one-half of the amount of sodium hydroxide needed to form the sodium salt, was fed. Only half of the glycine was made into the salt because the amount of sodium hydroxide necessary to accomplish this is about equal to that required to form the sodium salt of leucine and the monosodium salt of glutamic acid. While the ionization and the osmotic pressure of these solutions of the sodium salt of glycine probably differed from those of the sodium salts of leucine or of glutamic acid, it was felt that the way in which the salt tended to act might be determined.

The results of this experiment are shown in Table III. The rate of absorption is clearly higher than for glycine, and, with the exception of the 1 hour period, quite constant, the average being 64.2 mg. as compared with 50.3 mg. per 100 gm. per hour, the value found for glycine.

*dl-Alanine.*⁶—*dl*-Alanine was fed to fourteen rats, the time allowed for absorption ranging from 2 to 4 hours. As will be seen in Table IV, the absorption rate found was considerably higher than was the case with glycine; the average value was 73.6 mg. per 100 gm. per hour, with values varying from 45 to 92 mg., the determinations being much less constant than those obtained

⁶ Obtained from the Eastman Kodak Company and the Pfanstiehl Company.

with glycine or its salt. In spite of this variation, the values, with only two exceptions, are higher than the highest value found for glycine in a 2 to 4 hour absorption period.

d-Alanine.⁷—Because of the high cost of this material an extended series of experiments was not possible. However, we were

TABLE III.
Rate of Absorption of Glycine Fed as the Sodium Salt.

Rat No.	Sex.	Weight after fast.	Absorption time.	Glycine fed.	Glycine recovered.	Rate of absorption.
		gm.	hrs.	mg. per 100 gm.	mg. per 100 gm.	mg. per 100 gm. per hr.
39	M.	170.0	1	187	102	85
42	"	187.0	1	152	73	79
49	"	227.0	1	158	118	40
Average.....						68.0
41	F.	152.0	2	192	65	64
43	"	143.0	2	199	83	58
47	M.	202.5	2	177	59	59
48	"	203.5	2	179	52	64
111	"	117.0	2	489	344	73
Average.....						63.6
112	M.	137.5	3	416	224	64
114	"	141.0	3	405	219	62
Average.....						63.0
113	M.	148.0	4	386	155	58
Average of all experiments.....						64.2
" " 2, 3, and 4 hr. experiments.....						62.8

able to determine the rate of absorption of this acid over a 3 hour period with six rats. As will be seen in Table IV, with one exception, the determinations were very uniform, the rate of absorption being found to be practically the same as that of the racemic acid.

⁷ Obtained from the Hoffmann-LaRoche Company.

TABLE IV.
Rate of Absorption of dl-Alanine and d-Alanine.

Rat No.	Sex.	Weight after fast.	Absorption time.	Alanine fed.	Alanine recovered.	Rate of absorption.
<i>dl-Alanine.</i>						
		<i>gm.</i>	<i>hrs.</i>	<i>mg. per 100 gm.</i>	<i>mg. per 100 gm.</i>	<i>mg. per 100 gm. per hr.</i>
67	M.	128.5	2	233	65	84
70	"	146.0	2	364	249	58
94	"	109.0	2	352	169	92
96	"	117.5	2	323	173	75
Average.....						77.3
66	M.	108.5	3	276	28	83
68	"	109.5	3	287	96	64
71	"	134.0	3	394	259	45
72	"	100.5	3	529	263	89
95	"	115.0	3	334	134	67
97	"	107.0	3	355	107	83
116	"	149.0	3	271	47	75
118	"	162.0	3	245	42	68
Average.....						71.8
115	M.	129.0	4	312	10	76
117	"	123.5	4	321	36	71
Average.....						73.5
" of all experiments.....						73.6
<i>d-Alanine.</i>						
157	M.	104.5	3	303	30	91
159	"	113.5	3	279	43	79
160	"	108.0	3	294	70	75
161	"	130.5	3	290	68	74
162	"	133.5	3	284	73	70
163	"	128.5	3	295	63	77
Average.....						77.7

dl-Alanine as the Sodium Salt.—For the reason already discussed, *dl*-alanine to which half of the theoretical amount of sodium hydroxide had been added was fed. Of the fifteen rats

used, seven had to be discarded, as alanine, when fed as the salt, produced a marked diarrhea in a short time. For the same reason all of the values which were obtained were for a fairly short period, only two experiments being successful for a 3 hour period. The average rate of absorption (Table V) was found to be a value decidedly lower than was found for *dl*-alanine, in marked contrast to the rise in absorption rate when the sodium salt of glycine was fed in place of glycine.

TABLE V.
Rate of Absorption of dl-Alanine Fed as the Sodium Salt.

Rat No.	Sex.	Weight after fast.	Absorption time.	Alanine fed.	Alanine recovered.	Rate of absorption.
		gm.	hrs.	mg. per 100 gm.	mg. per 100 gm.	mg. per 100 gm. per hr.
143	M.	128.0	2	301	224	39
146	F.	138.0	2	277	176	51
153	M.	146.0	2	271	164	54
164	"	174.0	2	212	115	48
166	"	192.0	2	195	132	32
167	"	138.5	2	264	171	47
Average.....						45.2
140	M.	140.5	3	341	190	50
144	"	117.0	3	329	181	49
Average.....						49.5
" of all experiments.....						46.3

l-Leucine.⁸—*l*-Leucine was fed as the sodium salt for absorption periods of 2 to 4 hours. Although there was considerable variation noticed (Table VI), the results are unquestionably lower than those found for the other amino acids studied, the values ranging from 25 to 57 mg., with an average of 42 mg. of leucine absorbed per 100 gm. per hour.

d-Glutamic Acid.⁹—The feeding of the monosodium salt of *d*-

⁸ The leucine (Pfanstiehl) dissolved in 20 per cent hydrochloric acid showed a specific rotation of +20.3° as compared with the value of +15.8° obtained by Fischer and Warburg (6).

⁹ The glutamic acid was prepared from Ajinomoto in this laboratory.

glutamic acid gave varying rates of absorption (Table VII). Since this amino acid has a rather high molecular weight and a low nitrogen content, a slight variation in the residual nitrogen of the intestinal tract would produce a considerable change in the calculated absorption rate. The values are so high in some instances, however, that it is very doubtful whether this can be the complete explanation. The small number of experiments for the

TABLE VI.
Rate of Absorption of l-Leucine Fed as the Sodium Salt.

Rat No.	Sex.	Weight after fast.	Absorption time.	Leucine fed.	Leucine recovered.	Rate of absorption.
		gm.	hrs.	mg. per 100 gm.	mg. per 100 gm.	mg. per 100 gm. per hr.
78	M.	164.0	2	192	143	25
81	"	175.5	2	183	88	48
82	"	174.0	2	166	72	47
Average.....						40.0
79	M.	111.5	3	288	116	57
80	"	152.0	3	207	105	34
84	"	158.0	3	182	63	40
103	"	134.0	3	234	126	36
105	"	100.5	3	313	165	49
Average.....						43.2
104	F.	111.5	4	282	126	39
106	M.	115.5	4	273	89	46
Average.....						42.5
" of all experiments.....						42.1

shorter periods of absorption makes it impossible to reach any definite conclusion regarding the absorption rate immediately after the ingestion of glutamic acid, but it appears that after an initial very rapid absorption, there is a very decided drop so that according to the averages in Table VII, the absorption has decreased from 102.3 mg. per 100 gm. during the 1st hour to 8.7 mg. between the 2nd and 3rd hours, with an increase again to 76.9 mg. between the 3rd and 4th hours. However, the wide variations

found during the first 2 hours make the average value for these periods of very little significance. While it is doubtful if there

TABLE VII.

Rate of Absorption of d-Glutamic Acid Fed as the Monosodium Salt.

Rat No.	Sex.	Weight after fast.	Absorption time.	Glutamic acid fed.	Glutamic acid recovered.	Rate of absorption.
		gm.	hrs.	mg. per 100 gm.	mg. per 100 gm.	mg. per 100 gm. per hr.
30	M.	187.0	1½	264	187	84
51	F.	169.5	1	360	244	116
52	"	168.5	1	361	254	107
Average.....						102.3
34	M.	179.5	2	277	163	57
35	"	162.0	2	307	136	85
50	F.	146.5	2	416	222	97
55	M.	224.5	2	285	78	104
Average.....						85.8
31	M.	170.0	3	293	135	53
33	"	143.0	3	344	149	65
54	F.	173.0	3	370	195	58
127	M.	105.0	3	487	265	74
130	"	167.5	3	308	125	61
132	"	126.0	3	326	157	56
133	"	167.5	3	243	81	54
Average.....						60.1
128	M.	132.0	4	390	100	73
129	"	150.5	4	340	107	58
131	"	129.0	4	319	70	62
134	"	138.0	4	295	40	64
Average.....						64.3
" of all experiments.....						73.8
" " 3 and 4 hr. experiments.....						61.6

is as extreme a drop as this with a subsequent increase, it does seem as if there may be a greater initial rate than is found later.

TABLE VIII.

Rate of Absorption of Amino Acids. A Comparison of the Methods of Calculation on the Basis of Unit of Body Weight and Unit of Body Surface.

Substance fed.	Absorption period.	No. of experiments.	Absorption.					
			Amino acid.	N		Amino acid.	N	
			mg. per 100 gm. per hr.	mg. per 100 gm. per hr.	per cent	mg. per 100 sq. cm. per hr.	mg. per 100 sq. cm. per hr.	per cent
Glycine.	1	3	66.37±22.30	12.39	33.6	36.80±12.44	6.87	33.8
	2	4	52.28± 2.87	9.76	5.5	30.05± 1.48	5.61	4.9
	3	6	50.92± 3.54	9.51	7.0	28.80± 2.29	5.38	8.0
	4	4	47.15± 2.67	8.80	5.7	26.38± 1.81	4.93	6.9
	1-4	17	53.08± 5.52	9.91	10.4	29.94± 3.41	5.59	11.4
	2-4	14	50.23± 3.06	9.38	6.1	28.46± 1.97	5.31	6.9
Glycine (Na salt).	1	3	68.17±15.46	12.73	22.7	39.30± 9.91	7.34	25.2
	2	5	63.40± 2.78	11.84	4.4	37.02± 1.38	6.91	3.7
	3	2	63.00± 0.96	11.76	1.5	35.95± 0.43	6.71	1.2
	4	1	57.80	10.79		33.50	6.25	
	1-4	11	64.12± 5.72	11.97	8.9	37.13± 3.44	6.93	9.3
	2-4	8	62.60± 2.34	11.69	3.7	36.31± 1.18	6.78	3.3
dl-Alanine.	2	4	77.22± 8.31	12.14	10.8	42.78± 4.23	6.73	9.9
	3	8	71.43± 7.73	11.23	10.9	39.19± 3.90	6.17	9.9
	4	2	73.35± 2.15	11.54	2.9	40.70± 1.43	6.40	3.5
	2-4	14	73.36± 6.83	11.55	9.3	40.43± 3.41	6.35	8.4
d-Alanine.	3	6	77.70± 3.60	12.22	4.6	42.43± 1.60	6.67	3.8
dl-Alanine (Na salt).	2	6	44.87± 4.88	7.06	10.9	25.87± 3.58	4.07	13.8
	3	2	49.85± 0.33	7.85	0.7	27.95± 0.72	4.40	2.6
	2-3	8	46.11± 4.03	7.25	8.7	26.39± 2.26	4.15	8.6
l-Leucine (Na salt).	2	3	39.70± 8.32	4.24	21.0	22.97± 5.05	2.46	22.0
	3	5	43.30± 6.06	4.63	14.0	24.24± 2.84	2.58	11.7
	4	2	42.35± 3.29	4.53	7.6	23.20± 1.79	2.48	7.7
	2-4	10	42.03± 5.24	4.49	12.5	23.65± 2.82	2.53	11.9
d-Glutamic acid (Na salt).	1	3	102.67±10.00	9.78	9.8	58.13± 6.63	5.53	11.4
	2	4	85.55±11.33	8.15	15.6	50.38± 7.22	4.80	14.4
	3	7	60.19± 4.05	5.73	6.7	34.50± 1.92	3.28	5.6
	4	4	64.10± 3.27	6.10	5.1	36.40± 1.71	3.47	4.7
	1-4	18	73.77±11.63	7.03	15.8	42.39± 6.87	4.04	16.2
	3-4	11	61.61± 3.76	5.86	6.1	36.19± 1.85	3.35	5.3

Body Surface in Relation to Rate of Absorption.

Pierce, Osgood, and Polansky (7) computed the rate of absorption of glucose on the basis of unit of body surface as well as unit of body weight. While their results did not indicate any notable advantage of either method of calculation in the matter of uniformity, they considered them slightly better when calculated on the basis of body surface. It was accordingly decided to calculate in both ways in this study to see if any more definite conclusion could be reached than was obtained by the above authors.

For the computation of body surface, the usual formula of Meeh, $S = kW^{\frac{2}{3}}$, was used. Benedict and MacLeod (8) have recently reviewed the literature concerning the value of k for the white rat and have concluded that the older Rubner constant (9.1) is most nearly correct,¹⁰ a conclusion supported by the more recent direct measurements of Lee (9). We have used the Rubner constant in our own study.

A duplication of the preceding tables, changed only in respect to the basis of calculation, is hardly necessary. In Table VIII a summary of the rates of absorption of the amino acids studied is presented, the rates being calculated in the two ways and the percentage variations compared. A study of Table VIII shows that neither method of calculation has much of an advantage over the other in the case of rats, confirming the findings of Pierce, Osgood, and Polansky (7) for carbohydrates. Table VIII contains in addition to the above, the rates of absorption of the amino acids as calculated on the basis of nitrogen absorbed, thus giving a comparison of the number of molecules of the acid absorbed per unit of time. On this basis the amino acids can be arranged in the following order of decreasing rates of absorption: *d*-alanine, glycine (Na), *dl*-alanine, glycine, *dl*-alanine (Na), *d*-glutamic acid (Na), and *l*-leucine (Na).

DISCUSSION.

A general survey of the work of other investigators indicates that there exists a considerable difference in the rates of absorption and catabolism of the various amino acids. There is quite general agreement that glycine and alanine are absorbed more

¹⁰ Benedict and MacLeod (8), pp. 360-361.

rapidly than the other acids (Folin and Denis (10), Levene and Kober (11), Levene and Meyer (12), Seth and Luck (13), Johnston and Lewis (1)). It should be remembered, however that all of the evidence is indirect, being based on blood and urine analyses, and this involves a second variable, the ease of disposal of the amino acids by the tissues. That the methods used can be said to give a fairly reliable picture of relative rates of absorption has been demonstrated in this paper, since the results obtained here (*on the basis of nitrogen*, the basis on which all of the former work was done) show that the rates of absorption of glycine and alanine are clearly greater than those of leucine and glutamic acid.

We are unable to agree with Bang (14) concerning the rate of absorption of leucine. While he found no increase in the rest nitrogen of the blood within 10 hours after feeding leucine, he did report an increase in blood urea nitrogen of 12 to 14 mg. per 100 cc. indicating a fairly rapid absorption with immediate deamination. The work of Seth and Luck has also shown a small increase only in the amino acid nitrogen, but it did not show a marked rise in urea nitrogen, their maximum rise in this blood constituent being less than 5 mg. per 100 cc., which indicates a slower absorption such as we have shown actually exists in rats.

The most serious disagreement between this work and that of other investigators is in the rate of absorption of alanine. Cori (15) reported a series of experiments in which he found the rate of absorption of glycine to be 0.048 gm. and of alanine, 0.045 gm. per 100 gm. per hour.¹¹ While the absorption rate of glycine as found by us (50 mg. per 100 gm. per hour) is in agreement with that given by him, we find a distinctly higher rate for alanine (73 mg. per 100 gm. per hour), a rate which is greater than that of glycine not only when considered in terms of the acid absorbed, but even when expressed in terms of nitrogen. As was indicated in the experimental part, there was considerable variation noted in the rate of absorption of alanine by different animals. However, of the fourteen rats used, only one had a rate as low as the

¹¹ Cori's paper is a preliminary note and full details of his work are not given. If he had used the sodium salt of alanine (as he may have done, although from the wording of the paper this hardly seems probable) his results for alanine (45 mg. per 100 gm. per hour) would agree with ours.

average reported by Cori, and only two rates were lower than the highest obtained for glycine in a 2 to 4 hour absorption period.

This higher rate of absorption explains as well as, or perhaps better than, the lower value, some of the findings previously observed. While the amino acid nitrogen of the blood rises sharply whenever glycine or alanine is fed, the urea nitrogen figures sometimes show a difference. Seth and Luck (13) in experiments with rabbits, recorded the amino acid and urea nitrogen values of the blood after the ingestion of amino acids. The sum of the rises in the amino acid and the urea nitrogen values was slightly greater after alanine than after glycine ingestion. In the work presented by Johnston and Lewis (1), the sum of these rises over the first 3 hours after the feeding of glycine was about the same as was found after alanine. Since alanine has a lower content of nitrogen than has glycine, equal changes in nitrogen would indicate a greater absorption of alanine. On the other hand, Johnston and Lewis found a greater rise in the non-protein nitrogen after glycine than after alanine, a finding which suggests a slower absorption of the alanine nitrogen. Seth and Luck also found that neither the urea nitrogen nor the amino acid nitrogen of the blood rose as much after the injection of alanine into an intestinal loop of a dog as was observed when glycine was used.

In all studies involving changes in the nitrogen content of the blood, it must be borne in mind that the magnitude of these changes is determined not only by the rate of absorption from the intestine, but also by the rate of removal from, and addition to, the blood of nitrogenous substances by the tissues. Luck (16) found that the amino acid nitrogen content of the liver and muscles of a rat was not as great after alanine ingestion as it was after glycine. This would suggest either that the alanine was less readily absorbed by the tissues or that it was catabolized more rapidly. Adequate studies of the urea content of tissues after the ingestion of amino acids have not been made. The observations made by Johnston and Lewis led them to believe that glycine may have been deaminized more slowly than alanine.

The position of glutamic acid as intermediate, in the rate of its absorption (on the basis of equivalent amounts of nitrogen), between glycine and alanine on the one hand and leucine on the

other, is in agreement with the blood analysis experiments of other investigators. Johnston and Lewis concluded that in the rabbit the rate of absorption of glutamic acid was probably less than that of glycine or of alanine. Seth and Luck observed that while there was little, if any, rise in the amino acid content of the blood following the ingestion of glutamic acid, there was a steady and significant increase in the urea nitrogen, a rise not found after leucine feeding. These results suggest that the rate of absorption, in terms of nitrogen, of glutamic acid is less than that of glycine or of alanine and greater than that of leucine, a condition which we have shown to exist in the rat, at least for the longer absorption periods. Csonka (17) fed isoglucogenic quantities of glycine, alanine, and glucose to phlorhizinized dogs. His conclusion was that "the rapidity of the absorption and elimination of glucose ingested in phlorhizin glycosuria is almost the same as the rapidity of the absorption, deamination, synthetic sugar production, and the elimination of such sugar, after ingestion of isoglucogenic quantities of glycocoll or alanine." If glucose was excreted by these animals as soon as it entered the blood, the conclusion could be drawn that the time required for the absorption of the amino acids and the sugar formation from them must have been of the same order as the time required for the absorption of the glucose.

Cori (2, 18) found that glucose was absorbed by rats at a rate of about 0.2 gm. per hour. In rats, then, the rate of absorption of glucose is several times as rapid as is the rate of absorption of amino acids. There are several possible explanations which may explain the lack of correlation of these results with those observed by Csonka.

It is possible that the excretion of ingested glucose does not occur as soon as it is absorbed. If this were the case, the absorption and conversion of the amino acids to glucose might occur during the period of retention of the ingested glucose. It was shown by Csonka, however, that the excretion of the ingested glucose began almost immediately after ingestion, a fact that does not suggest a retention of any considerable amount.

It must always be remembered that observations obtained on one species of animal must be applied with caution to other species. It may therefore be possible that the rates of absorption

of glycine and alanine by the dog are similar to that of glucose. While this would be very different from findings with rats, it still exists as a possibility.

A third explanation of the equal rates of excretion of extra glucose after glycine, alanine, and glucose ingestion is that the extra glucose after amino acid administration may be in part due to a stimulation leading to a depletion of the sugar already present in the body rather than to a conversion of the amino acid to sugar. In this regard, it is interesting to note that those acids which stimulate metabolism to the greatest extent are, in many cases, the sugar-forming acids. There are, however, several strong arguments against this explanation of excretion of extra glucose because of a stimulation by the ingested amino acids. The amino acid which has the greatest specific dynamic action (phenylalanine) does not cause an excretion of extra sugar by the diabetic organism. A further argument against this explanation is that these amino acids lead to the excretion of a very uniform amount of extra glucose, an amount exactly corresponding to all of the carbon atoms in glycine and alanine.

One other possible explanation exists, one that, as far as we have found, has no evidence for or against it. The rats used for the absorption experiments were normal animals; the dogs used by Csonka were not. Is it possible that phlorhizin will so alter the intestinal wall as to make the rates of absorption of these two amino acids similar to that of glucose? If this were so, the results obtained by Csonka could be easily explained.

The statement made by Cori (15) that the absorption of glycine and alanine could be represented by a straight line curve has been borne out by the experiments presented here, although perhaps with reservations. It will be noticed by referring to Table VIII that, with the exception of glutamic acid, the hourly rates for each substance studied are much the same, whether the absorption period be of 2, 3, or 4 hours duration, but that there appears in the studies of most of the acids a slight gradual decrease in the rate with the time. This would indicate the possibility that a marked decrease in the absolute amount of the amino acid present in the intestine may cause a slight decrease in the rate at which it can be absorbed. In order to say definitely that such a decrease with longer periods of time actually exists, the number of experiments

would have to be greatly increased, as the change is apparently not great and the individual determinations overlap. The study would have to be a statistical one.

Again, in the more detailed tables it will be seen that often, although not always, there is a higher rate of absorption observed with the animals given the greatest amount of amino acid. So while there seems to be a tendency for a uniform absorption regardless of the absolute amount of substance present in the gastrointestinal tract, it would appear that the absolute amount may exert some influence. It is evident from some recent work of Cori and Cori (19), that the rule of uniform absorption may not be applicable generally, since they observed with lactic acid and sodium lactate that the rate of absorption was greatly influenced by the amount fed.

In the experimental part it was mentioned that it was necessary to feed glutamic acid and leucine as salts. For this reason determinations were made of the rates of absorption of glycine and of alanine as their sodium salts so as to get some idea as to what differences might be expected because of the presence of the sodium ion. The results were found to be contradictory and the question is as far from being answered as before, except that it can be definitely stated that the amino acids, when fed as their sodium salts, may be absorbed at a rate quite different from that observed when they are fed as the free acids.

Cori and Cori (19) suggested that the greater absorption rate which they found when sodium lactate rather than lactic acid was fed, might be explained by the lower acidity of the sodium lactate. Since the addition of sodium hydroxide to alanine caused a decrease in its rate of absorption, this explanation could certainly not be applied here. That the much slower absorption of alanine as its sodium salt may be due to a certain toxic action of the compound is possible, as an acute diarrhea developed in so many of the rats, sometimes in a very short time. One rat, for instance, had a severe diarrhea in 15 to 30 minutes after being fed the alanine salt. An argument against the toxicity of this compound is that those animals which did not develop the diarrhea were fed as much as the others.

SUMMARY.

1. The rates of absorption from the intestinal canal of rats, of glycine, *dl*- and *d*-alanine, when fed as the free acids, and of glycine, *dl*-alanine, *d*-glutamic acid, and *l*-leucine when fed as the sodium salts, have been determined. The amino acids can be arranged in the following descending order of rate of absorption: *d*-alanine, *dl*-alanine, glycine (Na), *d*-glutamic acid (Na), glycine, *dl*-alanine (Na), and *l*-leucine (Na). In terms of equivalent amounts of nitrogen, the order is as follows: *d*-alanine, glycine (Na), *dl*-alanine, glycine, *dl*-alanine (Na), *d*-glutamic acid (Na), and *d*-leucine (Na).

2. The statement made by Cori that the rate of absorption is independent of the absolute amount and the concentration of the amino acid in the intestine, has been confirmed.

3. It has been shown that changes in the nitrogenous constituents of the blood after the ingestion of an amino acid can be partially explained by a consideration of the rate of absorption of the acid.

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COMPARATIVE STUDIES OF THE METABOLISM OF AMINO ACIDS.

III. THE FORMATION OF GLYCOGEN AFTER ORAL ADMINIS- TRATION OF AMINO ACIDS TO WHITE RATS.*

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That glycogen can be formed from protein in the animal organism has long been accepted (2). Pflüger in his classical monograph on glycogen (3) has pointed out many errors in the older work and has concluded: "Ich kann diese Auffassung nicht für falsch erklären, sie ist noch nicht bewiesen."¹ However, in 1910, after a long series of carefully controlled experiments (4), he concluded that protein was a possible source of glycogen. Important also are the observations of Osborne and Mendel (5) in which the glycogen contents of the bodies of rats maintained over long periods of time on diets entirely devoid of preformed carbohydrate were found to be similar although slightly less than those of control rats which received the usual starch-containing food mixtures.

With the acceptance of the theory that the behavior of protein in intermediary metabolism is determined by the chemical transformation of its component parts, the individual amino acids, it is evident that the question of the various amino acids as sources of glycogen becomes of great significance. It has long been recognized that, in the phlorhizinized dog, the conversion of certain amino acids to glucose may occur (6, 7). However, the demonstration that under the pathological conditions of experimental

* The material present in this and the preceding paper of this series (1) represents an abstract of part of the thesis presented by Robert Hugh Wilson in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

¹ Pflüger (3) p. 316.

diabetes, conversion to glucose is accomplished does not necessarily have important bearing in a consideration of the normal paths of metabolism of the amino acids in the organism. Similar criticism may be made of the application of the results of Mann and his coworkers (8) with hepatectomized dogs to the interpretation of normal metabolic phenomena.

The question of glycogen formation after the administration of amino acids to normal fasted animals has not been extensively studied. Cohn (9) in an attempt to establish the validity of the theory of carbohydrate formation from amino acids containing 6 carbon atoms observed that the livers of fasted rabbits which had received leucine orally contained more glycogen than did those of control animals. Simon (10) in a repetition of this work in which the animals were rendered glycogen-free by the use of strychnine and fasting, could find no evidence of glycogen formation either in the liver or in the rest of the body. Neuberg and Langstein (11) fed large amounts (20 to 30 gm.) of alanine to fasting rabbits and found 1 to 2 gm. of liver glycogen, from which they were led to consider alanine as a source of glycogen. No details are given nor are any control experiments cited. It can hardly be said from the data presented that the formation of glycogen from alanine has been definitely established, since liver values as high as those reported by Neuberg and Langstein have been observed by other investigators in rabbits fasted for several days without other treatment. Pflüger (12) fed glycine to two fasting dogs which had been depleted of glycogen by injections of phlorhizin. In one animal, no increase in liver glycogen was observed; in the other, 4.47 per cent glycogen was present. In two other experiments in which no phlorhizin was used liver glycogen values of 1.70 and 1.58 per cent were noted after glycine feedings.

Evidence that a mixture of amino acids could form glycogen when fed to a fasting dog was presented by de Corral (13) on the basis of changes in the respiratory quotient. The respiratory quotient was observed to be considerably higher 18 to 20 hours after feeding the amino acid mixture than was to be expected if protein were being oxidized, which was interpreted as evidence of the formation and combustion of glycogen.

Casein supplemented by alanine was found to be more effective in the formation of liver glycogen in white rats than was casein

alone (14), while if glutamic acid were fed with casein, glycogen formation appeared to be checked. Unfortunately no adequate control experiments on fasting animals were presented.

Thus it is evident that of the various investigations which are concerned with glycogen formation after the oral administration of amino acids, only two appear to have been well controlled, that of Pflüger (12) with glycine and that of Simon (10) with leucine. It was felt that these experiments might well be repeated and extended with animals in which the extent of the absorption of amino acids had also been studied (1) with uniform experimental conditions and with a series of animals sufficiently large to eliminate as far as possible errors due to individual variations. A possible relationship between the amount and rate of absorption of the amino acids and the changes in the glycogen content might exist.

Rats appear to be more suitable for the study of glycogen formation than are the common large experimental animals of the laboratory. Hens, rabbits, and dogs often show considerable amounts of glycogen after fasts of several days. The body of the rat, on the other hand, contains little glycogen at the end of a 24 hour fast and the fasting glycogen levels are much more uniform. Barbour, Chaikoff, Macleod, and Orr (15) found an average liver glycogen content of 0.16 per cent in white rats after a 24 hour fast with values ranging from 0.10 to 0.31 per cent in one series and from 0.08 to 0.48 per cent in a second series. Cori and Cori have reported two series of glycogen determinations in rats after 24 hour fasts. In the first series of sixteen animals (16) an average liver glycogen content of 0.20 per cent was observed, while in a second series of eight animals, the average value obtained was 0.10 per cent. Variations found by Cori and Cori were similar to those noted by the Toronto observers (15). After a 24 hour fasting period, Cori and Cori also noted that the glycogen content of the entire animal averaged 0.143 per cent of the body weight. These average figures and variations are similar to those we have obtained in the present series.

EXPERIMENTAL.

For this study of glyconeogenesis from amino acids, the same rats which were used for the study of the rate of absorption from

the intestine (1) were employed for the most part. The general technique for the preparation of the animals, *i.e.* the period of fasting and the oral administration of the amino acids, was the same as already reported, except that in all cases in which glycogen formation was studied, the preliminary fast was of 24 hours duration. At the end of the period of absorption under study, the animals were killed either by a blow on the head or with chloroform, the intestines were removed, the liver and the rest of the body weighed as quickly as possible and covered with a hot saturated solution of sodium hydroxide. In the earlier experiments, in order immediately to check glycogenolysis, the livers and bodies of the animals were frozen with liquid air. Later this was discontinued since it was found that in the time elapsing between the slaughter of the animal and the heating with the strong alkali, no noticeable destruction of glycogen occurred. The glycogen was precipitated from the hydrolyzed tissue by the addition of 2 volumes of 95 per cent alcohol. After careful washing, the precipitated glycogen was hydrolyzed with hydrochloric acid, the solution carefully neutralized with sodium hydroxide, and the glucose was determined by the method of Hagedorn and Jensen, checked in some cases by determination by the Munson and Walker modification of the Bertrand method.

In the experiments with fasted animals, to which no amino acids were fed, the rats, after the preliminary fasting period, received 2 cc. of water by stomach tube in place of the amino acid solution. The average values, 0.21 per cent in the liver with individual values ranging from 0.12 to 0.33 per cent, and 53 mg. per 100 gm. in the whole body with values ranging from 28 to 77 mg. were fairly uniform, particularly in the case of the liver. These values for liver glycogen agree well with those of Cori and Cori (16, 17) but the content of glycogen of the entire body as found by us was somewhat lower than the figure (0.143 per cent) found by them.

In later experiments with amino acids, it was desirable to feed the rats by stomach tube several times during the day, in an attempt to keep a supply of amino acids constantly present in the intestine in order to obtain a maximum absorption over a longer period. In order properly to control these experiments, rats were fed 2 cc. of water at 4 hour intervals and killed after 16 hours. No

significant increases in the glycogen content resulted, although in two of the seven experiments of this kind, the liver glycogen values were slightly increased.

In preliminary experiments, mixtures of protein (casein or gelatin) or glycine with butter² were placed in the cages and the rats were permitted to eat at will for 24 to 30 hours. Any change in the glycogen content of the animals was considered to be due to the effect of the protein or glycine since it has been shown pre-

TABLE I.

Glycogen Content of Rats When Fed Solid Protein or Glycine and Butter Oil.

Substance fed.*	Absorption time.	Glycogen.	
		Liver.	Entire body.
	hrs.	per cent	mg. per 100 gm.
Casein and butter.	29	1.67	220
	30	1.36	199
	30	1.97	228
Average.....		1.67	216
Gelatin and butter.	24	0.40	157
	25	0.72	143
	25	0.68	113
	26	1.86	149
	26	2.06	205
Average.....		1.14	153
Glycine and butter.	26	0.89	129
	25	0.77	150
Average.....		0.83	140

* In each of these mixtures the butter constituted 25 per cent of the weight, the protein or glycine the remainder.

vously (18) that under these experimental conditions the butter did not contribute significantly to the glycogen store. The results (Table I) show that these animals had a definitely higher percentage of glycogen than did the controls, thus confirming previous workers.

² Since it was impossible to secure consumption of the proteins or glycine alone, 25 per cent of purified butter oil was added. These mixtures were eaten well. It was difficult, however, to measure exactly the food consumed due to the loss by scattering.

TABLE II.

Glycogen Content of Rats after Feeding dl-Alanine, d-Alanine, and dl-Alanine as the Sodium Salt by Stomach Tube.

	Absorption time.	Amount absorbed.	Glycogen.	
			Liver.	Entire body.
	<i>hrs.</i>	<i>mg. per 100 gm.</i>	<i>per cent</i>	<i>mg. per 100 gm.</i>
<i>dl-Alanine.</i>	2	168	0.42	88
	2	115	0.36	93
	2	183	0.81	95
	2	150	0.37	84
Average.....			0.49	90
<i>dl-Alanine.</i>	3	248	0.28	60
	3	191	0.53	65
	3	135	0.29	66
	3	266	0.23	48
	3	200	1.38	108
	3	248	0.39	62
	3	224	1.07	
	3	203	0.87	
Average.....			0.63	67
<i>dl-Alanine.</i>	4	302	0.98	99
	4	285	0.47	77
Average.....			0.73	88
<i>dl-Alanine.</i>	15		1.58	165
	15		0.59	91
	16		1.21	147
Average.....			1.13	134
<i>d-Alanine.</i>	3	273	1.25	129
	3	236	0.73	89
	3	224	0.39	101
	3	222	0.43	76
	3	211	0.61	92
	3	232	0.63	103
Average.....			0.67	98
<i>dl-Alanine as the Na salt.</i>	2	107	0.33	78
	2	97	0.29	
	2		0.57	
	2	63	0.18	
	2	93	0.57	
Average.....			0.39	78

The glycogen values obtained after oral administration of the amino acids are presented in Tables II and III. Administration of alanine (*d*-, *dl*-, and the sodium salt of *dl*-alanine) resulted in

TABLE III.

Glycogen Content of Rats after Feeding Amino Acids by Stomach Tube.

Amino acid fed.	No. of animals used.	Time of absorption.	Amount absorbed, range.	Glycogen.			
				Liver.		Entire body.	
				Range.	Average.	Range.	Average.
		hrs.	mg. per 100 gm.	per cent	per cent	mg. per 100 gm.	mg. per 100 gm.
Glycine.	3	1-2	107-117	0.10-0.22	0.16	53-81	80
	5	3	127-167	0.10-0.29	0.20	50-82	61
	2	4	161-197	0.16-0.25	0.21	71-103	87
	5	15-16		0.13-0.46	0.26	29-100	69
Glycine (Na salt).*	3	1	40-85	0.14-0.17	0.15	41-58	50
	5	2	116-127	0.07-0.25	0.16	32-56	45
	2	3	186-192	0.20	0.20	68	68
	1	4	231	0.15	0.15	62	62
Leucine (Na salt).	3	2	49-94	0.15-0.21	0.17	50	50
	5	3	102-172	0.14-0.24	0.19	31-67	45
	2	4	156-184	0.21-0.24	0.23	31-70	51
	3	11-16		0.10-0.23	0.14	24-50	41
Glutamic acid (Na salt).	3	1	77-116	0.19-0.22	0.21	54-75	67
	4	2	114-207	0.10-0.39	0.25	35-77	51
	7	3	158-222	0.21-0.59	0.33	54-95	72
	4	4	233-290	0.15-0.33	0.22	48-70	62
	2	15-16		0.23-0.38	0.31	67-98	83

* Only half of the amount of sodium hydroxide necessary to neutralize the carboxyl groups of alanine or glycine was added (1). Leucine was fed as the sodium salt and glutamic acid as the monosodium salt. In the case of these last two amino acids, it was impossible to prepare solutions of the free amino acids sufficiently concentrated for the purposes of the experiments.

definitely higher levels of glycogen (Table II), both for the liver and for the total glycogen content of the body. The increases in the total glycogen content were due in part to the higher glycogen content of the liver, but in most cases, the rest of the body also

showed an increase. Thus in the control series, the absolute values for glycogen of the body without the inclusion of the liver ranged from 29 to 99 mg. with only ten of the values of a series of twenty-five exceeding 70 mg., while after the administration of *dl*-alanine, the absolute values ranged from 39 to 118 mg. with eleven values of a series of sixteen exceeding 70 mg.

The results obtained after the administration of the other amino acids studied (Table III) do not indicate any distinct changes in the glycogen content either of the liver or of the entire body. The negative results with leucine are in accord with the earlier work of Simon (10) and opposed to the results of Cohn (9) who observed deposition of glycogen after the administration of leucine.

After the ingestion of monosodium glutamate, certain of the values for liver glycogen were slightly but clearly greater than those of the control rats. There were also some low values, but these did not occur as frequently as in the control series. The figures for the glycogen content of the entire body were slightly greater than the average for the controls, but the difference was not marked. After the longer periods (16 hours) of absorption, the glycogen values were within the range found for control rats similarly treated. It may be noted that considerable difficulty was experienced in the absorption experiments with glutamic acid over these longer periods of time. In two experiments the rats died during the course of the period of absorption and a third appeared ill during the last 6 hours of the experiment. We have observed previously this toxic action of glutamic acid (19) and can offer no explanation for it.

Certain previous investigations have suggested that glyconeogenesis may occur more readily from alanine than from glycine or glutamic acid, although all three amino acids have been shown to give rise to urinary glucose in the phlorhizinized dog (6, 7, 20). Voegtlin, Dunn, and Thompson (21) believed that alanine was more rapidly converted to glucose than was glycine or glutamic acid. They fed rats, previously injected with minimal lethal doses of insulin, the amino acids by stomach tube. Ingested alanine alone was able to prevent insulin convulsions and the death of the animal. The protective action of glycine or of glutamic acid was slight. It was concluded that alanine was absorbed much more rapidly than were the other amino acids or

that it was more easily transformed to glucose. Alanine protected the insulin-injected rats almost as effectively as did glucose, which suggests similar rates of absorption. But, as previously discussed (1), glucose appears to be absorbed much more rapidly than alanine. It may be that with the larger amounts of alanine fed by Voegtlin and coworkers (6 to 7 gm. per kilo of body weight), the absorption of alanine was greater than that observed by us.

A difference in the amount of glucose which could be formed during a given period of time might be expected even if glycine and alanine were converted to glucose with equal ease. We have shown previously that alanine is absorbed more rapidly than glycine (1). On the assumption of a complete utilization of all 3 carbon atoms of alanine for glucose formation, the glucose formed from alanine would amount to 101 per cent of the weight of the alanine. On the other hand, if all the carbon of the glycine molecule is available for glucose formation (6), which has been denied by Cremer (22), the glucose formed would be equal to only 80 per cent of the weight of the glycine converted. Hence it follows that from glycine with an average rate of absorption of 50 mg. per 100 gm. of body weight per hour (1), the maximum amount of glucose formed per 100 gm. of body weight would be 40 mg. per hour, while from alanine with an absorption rate of 73.6 mg., glucose formation might occur at the rate of 74 mg. per hour per 100 gm. of rat. The same type of calculation for glutamic acid, which has been shown to form glucose in phlorhizin diabetes to the extent of 3 of its 5 carbon atoms (23) indicates that the maximum formation of glucose from this amino acid would be about 37 mg. per 100 gm. per hour. It is clear that, on a theoretical basis, the opportunity for the formation of glucose should be much greater after the ingestion of alanine than after either glycine or glutamic acid.

It is possible also that the increased metabolism occasioned by the administration of the amino acids (specific dynamic action) may also affect the deposition of glycogen. The increased metabolism is indicative of increased oxidation and since carbohydrate is the most readily oxidized of the organic foodstuffs, it might be expected that to furnish the energy for this increased metabolism, glucose, if available, would be burned. It has been found that alanine increases metabolism to the extent of about

69 per cent of the increase which results from the ingestion of an equal weight of glycine (24). Hence less of the glucose formed from alanine should be used for purposes of oxidation than would be the case after the ingestion of glycine. This would leave more glucose available for deposition as glycogen after the administration of alanine.

A consideration of these factors, more rapid absorption of alanine, the possible greater ease of its conversion to glucose, and its lower specific dynamic action, indicates that formation of glycogen from alanine might be expected to take place more readily than from glycine.

In conclusion it should be pointed out that the results of the present study are not to be considered as significant except over short periods of time. They indicate that the formation of glycogen occurs more readily from alanine than from glycine, glutamic acid, or leucine. Over longer periods of time with the continued ingestion of large amounts of the amino acids, as for example in the experiments of Pflüger (12), glycogen formation from other amino acids may well be observed. Further studies to include other amino acids and a wider variety of conditions are in progress.

SUMMARY.

1. The glycogen contents of the liver and the entire body except the abdominal viscera of rats fed casein, gelatin, or glycine with butter for 24 to 30 hours were higher than those of the fasted control rats.

2. The oral administration of *d*- and *dl*-alanine by stomach tube to fasting white rats resulted in a rapid deposition of significant amounts of glycogen. After similar administration of glycine and *l*-leucine, the glycogen contents were similar to those of the control animals. The ingestion of the monosodium salt of *d*-glutamic acid was followed by a slight increase in the glycogen content as compared with control animals.

3. The possible relationships of the rates of absorption and specific dynamic actions of the amino acids to their ability to serve as precursors of glycogen are discussed.

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